

Characterization of pesticide resistance in horn fly populations as a risk factor for fixation of resistance alleles in cattle tick populations

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Introduction

The false assumption that parasite control is easily accomplished by the use of chemical means alone has led to the development of parasite resistance. In Brazil, the losses related to infestations by cattle ticks and horn flies are substantial, since nearly all cattle raising regions are also favorable to the development of these parasites. The selection caused by chemical treatments increases the frequency of resistant individuals in the parasite population, with consequent reduced efficacy of pesticide products and level of control. The problem of resistance of cattle ticks and horn flies will tend to get worse in the short term because the chemical treatments targeting one species also impose selective pressure on other species, since both parasites infest the same host. Currently, molecular diagnostic tools used in epidemiological studies are fundamental to discover and measure the risk factors responsible for the establishment of resistant genotypes in parasite populations. Therefore, the aim of the proposed studies is to characterize the population dynamics and predominant genetic mechanisms of the pesticide resistance response in populations of *Haematobia irritans* and *Rhipicephalus microplus*, as well as to verify whether identification of genetic mutations conferring resistance to insecticides in horn flies can be used as an indicator to predict the appearance of resistance in tick populations. The early detection of resistance in horn flies can lead to the formulation of improved management strategies regarding the products used to control ticks, thus helping to delay the establishment of pesticide resistance in those populations.

Hypothesis

The horn fly and the cattle tick utilize the same host for their biological development and cattle herds are infected simultaneously by both parasite species. The horn fly has a shorter period of biological development (egg to adult) than the cattle tick and the treatment to control one of these parasites affects the other as well.

Starting from these premises, the hypotheses that will be tested in the project related to the control of these cattle parasites are:

1. Pesticide treatments targeted to control the horn flies affect the frequency of resistance alleles in cattle tick populations.
2. It is possible to use the horn fly as a sentinel species to prevent the establishment of pesticide resistance in cattle ticks.



Figure 1: Cattle infested by *Haematobia irritans* and *Rhipicephalus microplus*

Materials and Methods

Identification of horn fly populations susceptible to pyrethroid pesticides (phenotypic and genotypic): Samples of horn flies will be collected from cattle naturally infested by *H. irritans* in at least 10 herds established in the states of Rondônia and São Paulo. The animals' susceptibility will be determined by the filter paper assay (Sheppard and Hinkle, 1987). The flies will be exposed to insecticide impregnated filter paper and placed in Petri dishes. The mortality rate will be determined after 2 hours and the results will be analyzed using probit analysis to obtain the LC50. The resistance factor (RF) will be calculated using the formula $RF = LC50 \text{ of the population tested} / LC50 \text{ of the susceptible population}$. Samples of resistant flies will be deposited in cryogenic vials and frozen immediately. DNA extraction will occur in accordance with the method described by Li et al. (2002). The DNA from the pyrethroid-resistant flies evaluated by the bioassay described above will be subjected to polymerase chain reaction (PCR) according to the method proposed by Guerrero et al. (1997). **Identification of the cattle tick populations susceptible to pyrethroid pesticides (phenotypic and genotypic testing):** Strains of ticks susceptible to pyrethroid pesticides will be identified. Tick samples will be collected from cattle naturally infested with *R. microplus* in at least 10 tick populations established in the states of Rondônia and São Paulo. Two bioassays will be performed for the detector of resistance in the tick samples: a) adult immersion test (AIT) (Drummond et al., 1973), b) larval packet test (LPT) (Stone and Haydock (1962), as modified by Miller et al., 2003), which will use larvae from females submitted to AIT and that lay viable eggs, according to the methods described above. The data obtained in the tests will be used to determine the different reproductive parameters and LC50 and LC90. DNA samples obtained from individualized larvae and the DNA from pyrethroid-resistant larvae evaluated by the bioassay described above will be submitted to polymerase chain reaction (PCR) according to the method proposed by Guerrero et al. (2001). **Characterization of the establishment of alleles of resistance to pyrethroid pesticides in populations of the horn fly and cattle tick:** *R. microplus* larvae identified as homozygous susceptible will be artificially infested in cattle affected by susceptible populations of horn flies. Each animal will be infested with 1 g of newly hatched *R. microplus* larvae, which represents an initial infestation of 20,000 larvae/animal. The cattle will be isolated in pastures at least 2 km away from the other animals in herds established in experimental fields of Embrapa. Cattle infested with strains susceptible to pyrethroid pesticides will be treated three times a month for a minimum period of 18 months, with increasing doses of pyrethroids, calculated from the LC50 established for the population of *H. irritans*, with the aim of selecting flies and ticks with KDR type alleles of resistance in parasite populations. Before applying the pyrethroid solution to the animals, samples of flies and ticks will be harvested for phenotypic analysis (bioassays) and genotypic analysis (PASA-PCR), in order to follow the dynamics of the establishment of resistance to pyrethroid pesticides and observe the frequency of resistant KDR alleles in the horn fly and the cattle tick populations that are under selective pressure inducing resistance to this pesticide. Mortality data obtained from the bioassays (filter paper, LPT and AIT) will be analyzed using the SAS statistical software (SAS Institute Inc., 1996), to obtain the lethal concentration (LC50) for populations of horn flies and ticks. Resistance rates (RR) for the populations of *H. irritans* and *R. microplus* will be calculated by the ratio of the LC50 of selection pressure compared with susceptible strains.

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